Step-Down Gradient Improves the Chromatographic Separation of Sucrose Monocaprate Regioisomers

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Step-Down Gradient Improves the Chromatographic Separation of Sucrose Monocaprate Regioisomers

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Introduction

Sugar fatty acid esters have a broad range of industrial applications and are used in the food, cosmetic and pharmaceutical industries. These compounds can be synthesized by conventional chemical processes or by enzymatic methods in organic solvents. Their physical and chemical properties depend on fatty acid chain length and both position and degree of esterification. The sugar monoesters are the most important types of these compounds because of higher solubility in water compared to the corresponding oligoesters.

In analysing sugar fatty acid monoester syntheses, it is necessary to achieve separation of the regioisomers. Ritthitham et al. (2009) previously demonstrated that when using reversed-phase high-pressure liquid chromatography (RP-HPLC), a step down in the concentration of acetonitrile in the eluent, below the initial concentration, improved the separation of sucrose fatty acid monoesters significantly. They also provided the elution order for seven regioisomers of sucrose monocaprate. Sucrose, shown in Figure 1, has eight hydroxyl substituents, and can form up to eight monoester regioisomers.

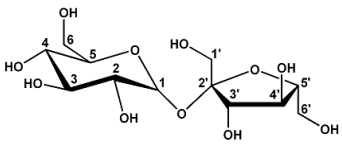


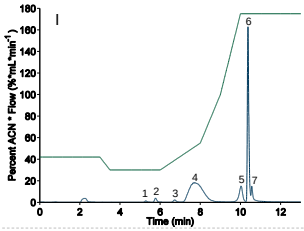
Figure 1: Haworth perspective formula of sucrose with carbon atom numbering.

Aim

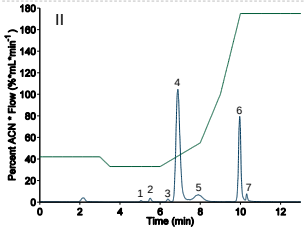
The aim of the present study was to improve the chromatographic separation of sucrose monocaprate regioisomers using reversed-phase high-pressure liquid chromatography.

Method

A commercial sample of sucrose monocaprate (>95 %) was analysed on an HPLC-system (HP Series 1100) with evaporative light-scattering detector (Alltech ELSD 800). The stationary phase was a C18 column (Waters Symmetry, 5 µm, 4,6x250mm) and gradients of acetonitrile (CH_3CN) in water (H_2O) were used as mobile phase.



Peak No.	Regio-isomer	Results		
		t_R	R_s	H
1	3'-*O*-	5.29	-	0.026
2	2-*O*-	5.77	2.1	0.025
3	4-*O*-	6.71	3.8	0.025
4	6-*O*-	7.73	1.3	0.48
5	3-*O*-	10.03	2.6	0.029
6	6'-*O*-	10.38	1.0	0.0077
7	4'-*O*-	10.58	0.78	0.0096



Peak No.	Regio-isomer	Results		
		t_R	R_s	H
1	3'-*O*-	5.04	-	0.028
2	2-*O*-	5.51	2.1	0.027
3	4-*O*-	6.39	3.7	0.022
4	6-*O*-	6.88	1.2	0.13
5	3-*O*-	7.92	1.3	0.23
6	6'-*O*-	9.96	2.9	0.030
7	4'-*O*-	10.31	1.0	0.0085

Figure 2: Chromatograms and elution profiles for elution programmes 1 and 2.
I) Elution Factors: A 42 %, B 3 min., C 30 %. II) Elution Factors: A 42 %, B 3 min., C 33 %. All other elution parameters equal.
In tables: t_R – retention time (min.), R_s – peak resolution, H – peak height.

Results

In the present investigation of sucrose monocaprate regioisomers analysed using RP-HPLC, a systematic study of the separation effects was performed. Three elution factors important for separation were identified:

A) Initial concentration of CH_3CN in the eluent mixture
B) Duration of the initial isocratic elution
C) Step-down concentration of CH_3CN in the eluent mixture

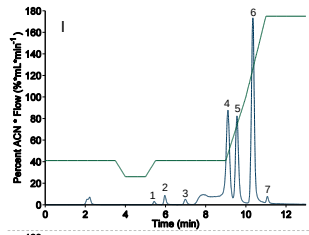
In one experiment elution factor C was varied while all other parameters were kept constant, while the effects of variation of elution factors A and B was investigated separately.

References

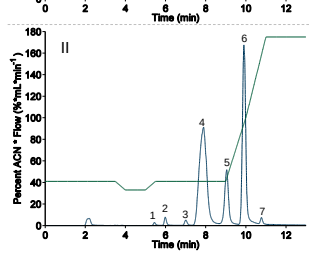
Ritthitham S, Wimmer R, Stensballe A, Pedersen LH. (2009) Analysis and purification of O-decanoyl sucrose regio-isomers by reversed phase high pressure liquid chromatography with evaporative light scattering detection. Journal of Chromatography A. 1216(25):4963-4967

Figures 2 and 3 show that elution factor C has a significant effect on the separation of the sucrose monoester regioisomers. In figure 2, a change in elution factor C from 30 % to 33 % between elution programmes 1 and 2 resulted in retention times for individual regioisomers being reduced with up to 21 % (peak 4). In addition, the band broadening of individual regioisomers – expressed as the peak height (or *height equivalent to a theoretical plate*) – was reduced by a factor of up to about 3.5, or increased by a factor of up to about 7.9, with this change in elution factor C (peaks 4 and 5, respectively). Similar results were observed for the change in elution factor C between elution programmes 3 and 4, shown in figure 3.

The results also indicate how the other elution factors affect retention times. Elution factor A has an influence on the retention times of all the sugar monoester peaks. While the effect of elution factor C appear to be dependent on an interaction between elution factors B and C, as the change in elution factor B from 3.5 to 3 minutes significantly reduced the impact of changes in elution factor C on peaks 1-3 (compare figures 2 and 3).



Peak No.	Regio-isomer	Results		
		t_R	R_s	H
1	3'-*O*-	5.44	-	0.025
2	2-*O*-	5.97	2.2	0.029
3	4-*O*-	6.99	3.8*	0.024
4	6-*O*-	9.11	5.1*	0.058
5	3-*O*-	9.56	0.84	0.046
6	6'-*O*-	10.35	1.5	0.0038
7	4'-*O*-	11.08	1.9	0.0084



Peak No.	Regio-isomer	Results		
		t_R	R_s	H
1	3'-*O*-	5.46	-	0.024
2	2-*O*-	5.99	2.4	0.023
3	4-*O*-	7.01	4.0	0.024
4	6-*O*-	7.89	1.6	0.18
5	3-*O*-	9.05	1.7	0.053
6	6'-*O*-	9.91	1.7	0.037
7	4'-*O*-	10.77	2.3	0.010

Figure 3: Chromatograms and elution profiles for elution programmes 3 and 4.
I) Elution Factors: A 41 %, B 3.5 min., C 26 %. II) Elution Factors: A 41 %, B 3.5 min., C 33 %. All other elution parameters equal.
In tables: t_R – retention time (min.), R_s – peak resolution, H – peak height.
* These values do not take into account the possible additional peak from this elution programme.

Table 1: Elution factors and separation results for individual elution programmes

Elution Programme	Elution Factors (A-B-C)	Retention time separation of regioisomers**	Standard deviation of peak resolutions	Peak resolution target fit***
1	42-3-30	0.31	1.13	0.56
2	42-3-33	0.38	1.10	0.57
3*	41-3.5-26	0.40	1.59	0.64
4	41-3.5-33	1.22	0.93	1.28
Ritthitham et al.		0.23	1.28	0.81

* The chromatogram suggests the presence of an additional peak. This has not been taken into account for the calculation of these values.

** $\frac{1}{\sum_{i=1}^{n-1}\left\lvert \Delta t_{R_i} - \overline{\Delta t_R} \right\rvert}$, where $\Delta t_{R_i} = t_{R_{i+1}} - t_{R_i}$, $\overline{\Delta t_R}$ is the average of the retention time differences, and n is the number of regioisomer peaks

*** $\frac{x}{\sum_{i=1}^{n} \left\lvert R_{S_i} - 1.5 \right\rvert}$, where n is the number of peak resolutions, and x is the number of $R_S \geq 1.5$

For all the analyses reported here, the separation of the monoester regioisomers was improved compared to previous reports. The optimal elution programme achieved a 400 % improvement in the separation of the sucrose monocaprate regioisomer peaks – based on retention times. In addition, the optimal elution programme shows a reduction in the standard deviation of the peak resolutions, as well as an improvement in how close the peak reolutions are to the goal of 1.5 (baseline separation). In fact, all peak resolutions are above 1.5 for elution programme 4 for the studied sample.

Conclusions

- A step-down gradient can significantly improve the separation of sucrose monocaprate regioisomers in reversed-phase high-pressure liquid chromatography analysis.
- The improvement achieved in the separation of sucrose monocaprate regioisomers in terms of retention times was in the area of 400 %.